Environmental drivers of grazing effects on arbuscular mycorrhizal fungi in grasslands

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Abstract

Arbuscular mycorrhizal fungi (AMF), common symbiotic root-associated soil biota, play a key role in maintaining ecosystem function and stability. However, how AMF are affected by livestock grazing in grassland ecosystem is variable, and this uncertainty in mycorrhizal responses to grazing is mainly due to the context-dependent nature of the AMF symbiosis association. The effects of grazing on AMF are through grazing-induced changes in the environment experienced by the mycorrhizal fungi and this includes both plant and soil-related factors. Here, we introduce a conceptual framework that highlights potential drivers and mechanistic pathways through which long-term grazing impacts AMF. We focus on known drivers of AMF abundance and diversity that are expected to be influenced by grazing including plant above-ground biomass and diversity, nutrient availability (soil available nitrogen and phosphorus) and edaphic properties (soil organic carbon, soil pH, soil bulk density and soil moisture). Given that the

identity and relative importance of each of these drivers may differ across different ecosystems, the application of our framework of potential environmental drivers allows us to generalize, organize and help formulate hypotheses for testing the impact of various grazing management approaches on AMF. We also argue the herbivory-AMF relationship can be moderated by climate, seasonality and topography. We reviewed the current state of knowledge of livestock grazing impacts on AMF and discussed some of the reasons behind conflicting results among empirical studies. We also provide suggestions for future research aimed at improving experimental design to unravel underlying mechanisms determining the impacts of grazing on AMF.

Key words: Mycorrhizal fungi, AMF-plant interactions, grazing, livestock, soil nutrients

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are one of the most common root-associated soil biota, which influence plant productivity, sustain above- and below-ground biodiversity (Moora and Zobel, 2010; Chen *et al.*, 2016; Brundrett and Tedersoo, 2018; Yang *et al.*, 2018), and improve soil attributes vital for plant growth (Augé, 2004; Simard and Austin, 2010; Wagg *et al.*, 2014; Mardhiah *et al.*, 2016) in different ecosystems. Grasslands are among the most degraded ecosystems worldwide over the last 50 years, mainly due to excessive grazing which brought about widespread soil erosion, plant productivity and biodiversity loss, and a decline in ecosystem functioning and services (Conant, 2010; O'Mara, 2012; McSherry and Ritchie, 2013). However, how the ubiquitous symbiotic AMF are affected by livestock grazing remains unclear.

This lack of certainty on grazing impacts on mycorrhizas can be explained by the context-dependent nature of this symbiotic association (Hoeksema *et al.*, 2010; Smith *et al.*, 2010; Tao *et al.*, 2016; Alzarhani

45 *et al.*, 2019). The indirect effects of grazing on AMF are through grazing-induced changes to the
46 mycorrhizal environment, including both plant and soil related factors. Although previous studies cover a
47 range of abiotic and biotic factors at different spatial and temporal scales (Ba *et al.*, 2012; Wang *et al.*,
48 2014; van der Heyde *et al.*, 2017), it is still difficult to generalize on the relative importance of drivers
49 behind AMF community characteristics across different environments and grassland management
50 systems. Moreover, the herbivory-AMF relationship may be moderated by climatic, seasonal and
51 topographical effects (Soudzilovskaia *et al.*, 2015; Johnson *et al.*, 2016). Given that the identity and
52 relative importance of each driver differs across different ecosystems (Alzarhani *et al.*, 2019), the suite of
53 all interacting biotic and abiotic parameters which are affected by grazing and influence AMF community
54 needs to be included in the analysis. This work will assist in providing further understanding of how the
55 key drivers impact AMF and allow the development of models that generalize and help formulate
56 hypotheses on the impacts of various grazing management approaches on AMF.

57 In this review, we present a conceptual framework to explain the indirect effects of long-term livestock
58 grazing on AMF mediated by changes in mycorrhizal environment, thus providing insights into the
59 potential environmental drivers and mechanistic pathways by which grazing alters AMF functioning. We
60 focused on the current knowledge about the impact of grazing on AMF in long-term studies due to the
61 time it takes for ecosystems to respond to environmental perturbations (McSherry and Ritchie, 2013). We
62 also review current knowledge of livestock grazing effects on AMF and provide suggestions for future
63 research aimed at improving experimental design and unravelling underlying mechanisms. This would
64 assist in generalization and help produce a synthesis of the grazing effects on AMF, and identify different
65 pathways through which grazing might alter AMF communities.

66

2. Conceptual framework and potential drivers of long-term livestock grazing effects on AMF

The context-dependent nature of the mycorrhizal symbiosis association suggests that the indirect effects of grazing on AMF occur through changes to the soil and root environment in which the AMF is found. Our framework proposes that the potential drivers of AMF which are expected to be influenced by grazing include plant community biomass and diversity, soil nutrient availability (here, soil available nitrogen (N) and phosphorus (P)) and edaphic properties (soil organic carbon, soil pH, soil bulk density and soil moisture). Moreover, the herbivory-AMF relationship can be moderated by climate, seasonality and topography effects (Figure 1). The framework defines the underlying mechanisms by which these potential drivers may be acting and explores their effects on AMF function and community structure (Table 1).

2.1. Plant community (biomass and diversity)

Obligate AMF depend upon their host plant for receiving photosynthetic carbon and in return they provide a wide range of beneficial services for their associated plants (Moora and Zobel, 2010; Powell and Rillig, 2018). The effect of herbivory on mycorrhizal fungi can be predicted, based on how grazing influences plant above-ground biomass and diversity (Figure 1). Since there is a close link between above- and below-ground parts of ecosystems (Yang *et al.*, 2018), direct effects of grazing events on the above-ground plant community would explain the indirect effects of livestock herbivory on AMF abundance and diversity. Here, we discuss the impact of long-term livestock grazing on AMF brought about by changes in plant biomass and diversity.

2.1.1. Plant biomass

136 manipulated grazing experiment with seven grazing intensities, Li *et al.* (2017) showed that plant species
137 richness significantly decreased up to 50% at higher grazing intensities (greater than 4.5 ewes (female
138 sheep) ha^{-1}) suggesting that grazing intensity should not exceed this threshold in order to maintain plant
139 biodiversity at their experimental site. The negative effect of grazing on plant species diversity may be
140 also caused by the removal of palatable dominant plant species from the species pool (Table 1) (Wan *et*
141 *al.*, 2011; Li *et al.*, 2017). Wan *et al.* (2015) reported a U-shaped relationship between plant species
142 diversity and grazing intensity and a severe loss of palatable plant species at intermediate levels of grazing
143 intensity. However, the abundance of low palatable plant species remained unaffected by grazing
144 intensity (Wan *et al.*, 2015).

145 Loss of plant diversity leads to a decline in the range of below-ground plant root types and root exudates
146 and consequently decreases the variability of the soil/root resources for soil micro-organisms including
147 AM root associated fungi (Ba *et al.*, 2012; Epelde *et al.*, 2017). Indeed, AMF spores germination, hyphal
148 extension and root infection take place in the presence of root and root exudates (Smith and Read, 2008;
149 Tahat *et al.*, 2010). In addition, it has been reported that root exudates change soil nutrient availability by
150 priming decomposition of soil organic matter (Lambers *et al.*, 2008; Smith and Read, 2008). Given that
151 AMF species have distinct nutrient absorption strategies (Mendoza *et al.*, 2011a; Guo *et al.*, 2016; Powell
152 and Rillig, 2018), and that grazing can alter both plant below-ground biomass and soil resource availability
153 (Hao and He, 2019), the abundance and composition of AMF would be expected to change in response to
154 grazing (Ba *et al.*, 2012).

155 Grazing, on the other hand, may enhance plant community biodiversity at moderate level of grazing
156 intensity by suppressing dominance by the more competitive plant species with faster growth rates and
157 thus promoting the coexistence of less competitive species with lower growth rates (Komac *et al.*, 2015;
158 Pulungan *et al.*, 2019). Highest levels of plant diversity has been suggested as a consequence of

159 intermediate levels of grazing intensity where the plant community is dominated by palatable species
160 with sub-dominant unpalatable plant species (Wan *et al.*, 2015).

161 Interestingly, it has been shown that grazing is likely to have a positive feedback on the strength of the
162 association between AMF and plant community under light- to moderate-grazing, but with a negative
163 impact under heavy-grazing pressures (Ba *et al.*, 2012). A conceptual model proposed by Ba *et al.* (2012)
164 suggested under light to moderate grazing a positive feedback on AMF-plant associations would occur
165 through improved photosynthetic and regrowth capability of the plants followed by increased below-
166 ground carbon allocation to mycorrhizal fungi. In contrast, under heavy grazing, considerable above-
167 ground biomass loss and below-ground carbon limitation could lead to less mutualistic benefits between
168 AMF and plant. However, the AMF-plant community composition interactions under real grazing
169 conditions, particularly at the large scale, have not been sufficiently investigated. Experiments at the local
170 scale showed either positive effects (Ren *et al.*, 2018) or no relationship (van der Heyde *et al.*, 2017)
171 between AMF and plant biodiversity in grazed grasslands. The underlying mechanisms are unclear,
172 however, a couple of factors can explain the possible positive or negative outcomes of the grazing impact
173 on the interaction between plant and AMF, including:

174 I. **Host plant identity:** Grazing reduced mycorrhizal root colonization significantly, and the greatest
175 reduction was observed for the most preferred grasses compared with the least preferred ones
176 in a sheep grazed site in the Patagonian steppe (Cavagnaro *et al.*, 2019). Similarly, grazing and
177 host-plant species explained most variation in AMF spore community composition at different
178 sites in Yellowstone National Park, USA (Mendoza *et al.*, 2011b). Likewise, the intra-radical AMF
179 community composition was significantly shaped by interactive effects of host plant identity and
180 grazing in grasslands in central Germany (Vályi *et al.*, 2015). Moreover, a greater diversity of host
181 plants for AMF may facilitate the establishment of some host-specific AMF species, enhancing

AMF diversity (Stover *et al.*, 2018). These finding suggests that grazing and host plant identity impact interactively on the abundance and community composition of mycorrhizal fungi.

II. **Plant functional diversity:** it has been suggested that C4 grasses, non-nitrogen-fixing herbaceous dicotyledonous and woody plants are more responsive to mycorrhizal colonization compared with C3 grasses and plants associated with nitrogen-fixing bacteria (Hoeksema *et al.*, 2010). Furthermore, Lin *et al.* (2015) showed a significant increase in the competitive ability of N-fixing forbs and significant decrease in that of C3 grasses after AMF inoculation by synthesizing the results of 304 studies. Additionally, there is evidence that loss of plant functional groups reduced the abundance or biomass of below-ground community components in temperate grasslands (Chen *et al.*, 2016). Thus, plant functional diversity almost certainly plays an important role in determining the effects of grazing on AMF.

III. **Mycorrhizal dependence of dominant and sub-dominant plant species:** AMF in a plant community with mycorrhizal-dominant plant species decreases plant diversity by increasing competitiveness of the dominant plant species. In contrast, AMF can exert positive impacts on plant diversity in a community with sub-dominant mycorrhizal plant species (Mendoza *et al.*, 2011a; Ba *et al.*, 2012; Lin *et al.*, 2015; Yang *et al.*, 2018). Grazing effects on, either dominant, or sub-dominant, mycorrhizal plant species could, therefore, differentially impact on AMF communities.

IV. **AMF isolates and functional diversity (mycorrhizal traits):** AMF species are different in terms of their functions and traits; for example some AMF species are better at defending their host plant against pathogens, whereas others are better at improving plant productivity through nutrient uptake (van der Heijden *et al.*, 2006; Hoeksema *et al.*, 2010; Yang *et al.*, 2017; Ferlian *et al.*, 2018; Powell and Rillig, 2018). Grazing impacts would in this case favor those plant-AMF associations best-placed to respond positively to any competitive release caused by grazing.

206 Taken together, changes in plant community diversity caused by grazing would impact AMF
207 functioning via a range of pathways. The possible outcome of this grazing effect can be explained in
208 the context of factors including host plant and AMF species identity, grazing intensity and
209 environmental conditions. For example, grazing can enhance forbs with low net primary productivity
210 and thereby reduce soil carbon input in one region while it can increase encroachment by shrubs with
211 high net primary productivity and increase soil carbon input in another region (Piñeiro *et al.*, 2010).
212 In both cases, changing the above-ground plant community composition would alter the inter-
213 connected below-ground soil biota, thus affecting soil ecosystem functioning and the soil carbon
214 cycle.

215 ## 2.2. Soil nutrient availability

216 Soil nutrient availability can be influenced positively by livestock grazing (van der Heyde *et al.*, 2017; Egan
217 *et al.*, 2018) via dung and urine addition (Vertès *et al.*, 2019). Dung is rich in carbon and phosphorus and
218 urine is rich in nitrogen and potassium. However, negative effects of grazing on soil resources (Steffens *et*
219 *al.*, 2008; Wiesmeier *et al.*, 2009; Guo *et al.*, 2016) and null effects (Li *et al.*, 2008; Burke *et al.*, 2019),
220 depending on the balance between inputs and outputs in the system, have been also reported (Table 1).
221 For example, in a recent meta-analysis conducted in manipulated field grazing experiments in Chinese
222 grasslands, Hao and He (2019) showed that grazing reduced soil total N and P by 3.9% and 9.4%
223 respectively. Negative impacts of herbivory on soil resource availability occurs via defoliation, reduction
224 in forage production, litter accumulation, followed by further decreases in soil carbon and nitrogen
225 availability (Piñeiro *et al.*, 2010).

226 Both positive and negative impacts of livestock grazing on soil nutrient content change mycorrhizal
227 behavior and activities since the obligate symbiotic AMF rely on plants for their C in exchange for nutrients
228 such as P and to a minor extent N (Figure 1). Indeed, strong correlations between abundance and diversity

229 of AMF and soil nutrient availability, including N (Birgander *et al.*, 2014; Soudzilovskaia *et al.*, 2015; Binet
230 *et al.*, 2017; Soka and Ritchie, 2018), P (Wang *et al.*, 2014; Guo *et al.*, 2016; Faghihinia *et al.*, 2020), and
231 their interactions (Chen *et al.*, 2014) have been reported in grazed grassland ecosystems. Therefore,
232 grazing-induced change in soil nutrient availability is expected to affect AMF by changing the mycorrhizal
233 environment. However, the mechanisms and pathways of long-term grazing impacts on AMF community
234 through altering soil stoichiometry are unclear due to the many reported conflicting results. For example,
235 a long-term grazing-induced reduction in soil N and P was significantly positively correlated with AMF
236 diversity in typical meadow steppe in north of China (Guo *et al.*, 2016), whereas the grazing-induced
237 positive effects on soil N and P was not related to AMF abundance and diversity in grazed prairies in
238 Canada (van der Heyde *et al.*, 2017). Conflicting results can be partly explained by relative availability of
239 soil resources. It has been suggested that the relative availability of soil nutrients, particularly N and P,
240 determines the outcome of plant-AMF interaction (Hoeksema *et al.*, 2010; Yang *et al.*, 2016). Positive
241 mutualisms and higher rate of AMF colonization in nutrient limited grasslands is expected due to greater
242 host nutrient demands for regrowth and to compensate for tissue loss caused by grazing (Guo *et al.*, 2016;
243 Faghihinia *et al.*, 2020). Meanwhile, decreases AMF colonization was reported at very low nutrient
244 availability due to apparent competition with the host plant for the limited resources (Piippo *et al.*, 2011).

245 Grazing effects on AMF through the altering of soil resource availability may also depend on which
246 nutrient is most limiting in the ecosystem. The findings of a meta-analysis of mycorrhizal inoculation
247 experiments are consistent with the hypothesis that AMF inoculation is most beneficial to plant growth
248 in P-limited systems (tissue N:P > 16 in non-inoculated plants) rather than N-limited (tissue N:P < 14 in
249 non-inoculated plants) (Hoeksema *et al.*, 2010) (Table 1). Given that different grasslands types located in
250 different climate zones differ in terms of N (Piñeiro *et al.*, 2010) and P availability (Zhou *et al.*, 2017),
251 variable AMF responses to grazing in different ecosystems must be expected. For example, low values of
252 AMF root colonization has been reported in the lowland equatorial zone (−15° and 15° latitudes) with low

253 soil P availability due to the evolutionary strategy of plants to cope with P limitation through cluster root
254 development (Soudzilovskaia *et al.*, 2015). In contrast, AMF root colonization intensity was greatest at
255 sites with high soil nitrogen availability and with a continental climate at the global scale (Soudzilovskaia
256 *et al.*, 2015). Further field investigations at the global scale are needed to test these hypotheses and
257 provide more insight into the underlying mechanisms of grazing impacts.

258 Soil fertility expressed as a C:N ratio may be another important predictor which could be affected by
259 grazing (Piñeiro *et al.*, 2010; Mendoza *et al.*, 2011a) and translate to changes in the AMF community
260 (Soudzilovskaia *et al.*, 2015). As a general global pattern, Piñeiro *et al.* (2010) reported increased C:N ratios
261 in grazed compared to ungrazed sites. Interestingly, in a global review across 233 sites an unimodal
262 relationship was described between soil C:N and intensity of root colonization by AMF, peaking in
263 moderately-fertile soils with a soil C:N ratio of 11.8 followed by a decreasing trend (Soudzilovskaia *et al.*,
264 2015).

265

266 2.3. Edaphic properties

267 Grazing can impact the edaphic properties of the mycorrhizal environment. For example, as a general
268 pattern, grazing increases soil bulk density and decreases soil moisture, permeability and hence water
269 available for plant growth (Wiesmeier *et al.*, 2009; Piñeiro *et al.*, 2010; Hao and He, 2019; Tang *et al.*,
270 2019b); this occurs where livestock trampling and treading leads to soil compaction, especially in dry
271 grasslands with lower water and nutrient availability. Moreover, by reducing the plant shading effect,
272 grazing exposes soil to the air, increasing soil temperature and reducing the water content (Table 1).
273 Considering the important role of soil water content in the plant-AMF relationship (Smith *et al.*, 2010;
274 Augé *et al.*, 2015), adverse effects of grazing on soil moisture will impact on AMF functioning. Indeed,
275 strong positive relationships between soil water content and AMF spore richness (Murray *et al.*, 2010;

276 Mendoza *et al.*, 2011b), AMF root colonization (Faghihinia *et al.*, 2020) and external hyphal density (Ren
277 *et al.*, 2018; Soka and Ritchie, 2018) have all been reported for grazing experiments. Grazing-induced
278 water limitation may reduce plant photosynthesis rates, and hence primary production, resulting in lower
279 N and P demands by the plant and lower availability of photosynthate C for allocation to fungal partners
280 (Mendoza *et al.*, 2011b).

281 Grazing can also exert either negative or positive effects on soil pH which also impacts on the mycorrhizal
282 environment (Table 1). Grazing may cause increases in soil pH via accelerating soil alkalization and
283 salinization processes through loss of above-ground vegetation cover, increasing soil temperature,
284 evaporation, and decomposition of soil organic matter (Kusakabe *et al.*, 2018). In contrast, grazing may
285 also cause soil acidification and decreases in soil pH via urination and nitrification of urine-N, especially
286 under high grazing pressure (Martins *et al.*, 2014).

287 Changes in soil pH influences the soil carbon and nutrient cycles (particularly N) thereby affecting the
288 activities of soil microorganisms involved in the transformations of compounds containing these two
289 elements (Kunhikrishnan *et al.*, 2016). Indeed, field evidence for positive relationships between pH and
290 AMF spore density (Mendoza *et al.*, 2011a; Birgander *et al.*, 2014; Soka and Ritchie, 2018), soil hyphal
291 length density (Hu *et al.*, 2013; Ren *et al.*, 2018) and AMF Shannon diversity in soil and root (Guo *et al.*,
292 2016) have been recently documented in grasslands.

293 At high soil pH, P can precipitate with calcium forming calcium phosphates and thus become unavailable
294 for plant uptake directly or via their associated microorganisms (Gupta *et al.*, 2008). Low soil pH, on the
295 other hand, has been frequently shown to drive AM fungal community structure (Mendoza *et al.*, 2011a;
296 Chagnon *et al.*, 2013). However, low pH, beyond a certain threshold, can cause P immobilization due to
297 fixation by aluminum and iron ions, also reducing its availability to plants, and thus increasing plant
298 requirement for interactions with AMF (Zhu *et al.*, 2007; Klichowska *et al.*, 2019).

299 Additionally, AMF species differ in terms of their optimal pH ranges (An *et al.*, 2008; Chagnon *et al.*, 2013)
300 and grazing-caused changes in AMF niche space could impact the abundance and community composition
301 of the mycorrhizal fungi (Wang *et al.*, 2014; Xu *et al.*, 2016). For example, a strong negative correlation
302 between AMF richness and phylogenetic diversity and pH was reported along a 5000-km transect at 47
303 sites with mostly alkaline soils and four different vegetation types including meadow, typical steppe,
304 desert steppe and desert in northern China (Xu *et al.*, 2016). The strong negative correlation were
305 attributed to disappearance of some AMF species under extreme soil alkaline condition (Xu *et al.*, 2016).

306 Grazing has also been suggested to impact on SOC content via three main pathways simultaneously, by
307 altering: (1) plant net primary productivity, (2) soil nitrogen stocks (SON), and (3) SOM decomposition
308 (Piñeiro *et al.*, 2010). Grazing may decrease SOC by reducing above-ground plant biomass, infiltration
309 rates, enhancing soil compaction and SOC decomposition particularly in C3-dominated temperate
310 grasslands (McSherry and Ritchie, 2013; Hao and He, 2019) (Table 1). Conversely, grazing may increase
311 SOC via enhancing soil nitrogen stocks (SON), plant tissue deposition, and below-ground root litter
312 deposition, particularly in C4-dominated subtropical grasslands (McSherry and Ritchie, 2013; Wilson *et*
313 *al.*, 2018). In either case, carbon allocation to roots and root exudates, and the abundance and activity of
314 soil micro-organisms including AMF, would be affected by grazing (Wilson *et al.*, 2018). In general, the
315 dynamic of grazing effects on AMF through changes in SOC, below-ground carbon allocation or root
316 exudation has not been addressed adequately in previous studies. However, it can be hypothesized that
317 lower carbon allocation to roots, root and microbial biomass, and lower SOC/SON stocks would negatively
318 impact the AMF abundance and activity under grazing. In contrast, grazing-induced increase in root
319 carbon allocation, fine root exudation and SOC/SON stocks could reduce AMF functioning. Having said
320 that, the effects of grazing on SOC is controlled by plant community productivity and composition, soil
321 nutrient content and precipitation (Piñeiro *et al.*, 2010; McSherry and Ritchie, 2013; Wilson *et al.*, 2018)
322 suggesting that all these factors, interacting together, will also influence the soil microbial processes.

Taken together, these finding demonstrate that changes in the edaphic properties is another pathway through which livestock can influence the environment experienced by mycorrhizas. It is worth, therefore, considering that all grazing-caused changes in physical and chemical properties occur simultaneously and that including them in frameworks and models could help to unravel the co-occurring mechanisms affecting mycorrhizal communities in different environment and better understand the grazing effects on below-ground processes.

2.4. Grazing-effects moderators

There is evidence that grazing effects on AMF community can be moderated by climate (Dickie *et al.*, 2013; Soudzilovskaia *et al.*, 2015; Zhang *et al.*, 2019), seasonality (Lingfei *et al.*, 2005; Regan *et al.*, 2014), topography (Ren *et al.*, 2018; Faghihinia *et al.*, 2020) and grazing intensity (Mendoza *et al.*, 2011b; Ba *et al.*, 2012; Ren *et al.*, 2018).

2.4.1. Climate

The latitudinal gradients in climate variables, mainly temperature and precipitation, has generated various types of grasslands with distinct floristic composition and abiotic features in different climatic zones (Faber-Langendoen *et al.*, 2012; Dixon *et al.*, 2014). Therefore, the possible outcomes of interacting effects of climate and grazing on above- and below-ground communities would likely lead to different results depending on ecosystem type. For example, in a review study of 67 paired comparisons at the global scale, Piñeiro *et al.* (2010) revealed that below-ground root biomass and SOC were greater in grazed compared with ungrazed sites at the driest and wettest sites, but were lower at sites with intermediate precipitation (400 mm to 850 mm). Moreover, it has been demonstrated that grazing can buffer (Wang *et*

344 *al.*, 2019) or exacerbate (Tang *et al.*, 2019a) the response of plant communities to climatic variation due
345 to its important influences on plant biomass, community composition and soil nutrient dynamics.

346 Despite a great deal of effort in investigating the effects of climatic variables and grazing on above-ground
347 community and nutrient cycling (Post and Pedersen, 2008; Tang *et al.*, 2019a; Wang *et al.*, 2019; Lv *et al.*,
348 2020), little is known about their interacting effects on the below-ground community, particularly at the
349 global scale. Much of the work has been primarily conducted in mesocosm experiments through the
350 manipulation of temperature (Hawkes *et al.*, 2008; Zhang *et al.*, 2019), rainfall (Deveautour *et al.*, 2019),
351 or both (Sun *et al.*, 2013) at very local scales. Data collected from 233 sites worldwide revealed the key
352 role of climate in explaining the global pattern of AMF root colonization (Soudzilovskaia *et al.*, 2015), for
353 example, showing that plant root colonization by AMF peaked at sites with warm growing season
354 temperatures and declined at sites with cooler or hotter growing seasons. However, how AMF are
355 influenced by grazing and climate in conjunction remains unclear. Given that grazing is a common practice
356 in almost all climatic zones, gaining an insight into how climate interacts with grazing impacts on AMF
357 functioning has essential implications for sustainable grassland managements and predicting the
358 consequences of the future climate change scenarios.

359

360 **2.4.2. Seasonality**

361 In addition to annual variations in climatic factors, climatic seasonality is also expected to moderate the
362 grazing effects on above- and below ground community as a result of inter-annual variations in
363 temperature and precipitation (He *et al.*, 2010; Li *et al.*, 2010; Ramos-Zapata *et al.*, 2011; Omirou *et al.*,
364 2013; Wang *et al.*, 2014), vegetation growth and plant phenology (Sánchez-Castro *et al.*, 2012), plant
365 nutritional quality (Mysterud *et al.*, 2011) and nutrient availability (Smith and Smith, 2011; Wang *et al.*,
366 2014).

Despite the major changes that seasonal variation causes to plant phenological development and soil nutrient status, the interaction of seasonality and grazing effects on mycorrhizal fungi has not been addressed sufficiently, and the underlying mechanisms remain poorly understood. Many studies addressed the response of AMF to grazing at a single seasonal time point (Bai *et al.*, 2013; van der Heyde *et al.*, 2017). However, there appears to be a pronounced seasonal pattern in AMF responses to grazing, particularly in temperate systems; this has been described for mycorrhizal root colonization (Lekberg *et al.*, 2013; Hazard *et al.*, 2014; Regan *et al.*, 2014; Wang *et al.*, 2014; Cavagnaro *et al.*, 2018), fungal hyphal length density (Staddon *et al.*, 2003; Wang *et al.*, 2014) and AMF spore density (Bever *et al.*, 2001; Wang *et al.*, 2014). Additionally, Wang *et al.* (2014) found a significant interaction between grazing management and seasonality on AMF abundance and community composition.

AM fungal abundance and activity is expected to peak in summer because of the greater plant nutrient demand, rapid vegetative growth and root production. In addition, plants may need more nutrients to fund shoot regrowth, thus invest more in AM fungi during summer when grazing is usually most intense (Cavagnaro *et al.*, 2018). Consistent with this hypothesis, Cavagnaro *et al.* (2018) observed that AMF root colonization of both sheep preferred and non-preferred grass species was significantly higher in summer compared with autumn in a steppe grassland in Argentina. Similarly, Staddon *et al.*, (2003), Mandyam and Jumpponen (2008) and Wang *et al.* (2014) reported greater mycorrhizal root colonization in the summer, and lower during the autumn in grassland ecosystems. The same seasonal pattern has been reported for fungal hyphal length density in a grassland soil (Staddon et al., 2003). However, no seasonal variation in AMF colonization has also been reported between summer and winter in a coastal- sandy, temperate-grassland in Denmark (Lekberg *et al.*, 2013). Further research is needed to explain these different observations.

390 Together, the current knowledge suggests that the impact of grazing on AMF community may not be
391 particularly representative at a given seasonal time point. Therefore, if we are to understand the
392 mechanisms underlying grazing effect on AMF, seasonality should not be overlooked in studies of grazing
393 on AMF. This could have large implications for grassland management in term of the timing of grazing
394 within the growing season and helping with management decisions aimed at maintaining sustainable
395 grassland productivity and soil biodiversity.

396

397 **2.4.3. Topography**

398 Topographic relief influences habitat conditions via the creation of gradients in soil water and nutrient
399 availability and solar exposure (Schowalter, 2016). Furthermore, topography impacts animal behavior and
400 their distribution leading to an heterogeneous grazing intensity (Johnson *et al.*, 2016). Lower lying areas
401 tend to be grazed more heavily with deposition of greater loads of dung and urine. Areas at higher
402 elevation, in contrast, experience low- to moderate-grazing intensity with less nutrient deposition
403 (Johnson *et al.*, 2016). Therefore, topography, even at the local scale, alters soil resource availability (e.g.
404 soil moisture, soil organic carbon and total nitrogen stocks) thereby affecting plant community structure
405 leading to changes in soil microbial biomass and composition (Murray *et al.*, 2010; Zhang *et al.*, 2013; Ren
406 *et al.*, 2018).

407 Given that the plant-AM fungi association is fundamentally a symbiotic relationship based on nutrient
408 exchange (Johnson et al., 2015; Powell and Rillig, 2018), topographic gradients of moisture and nutrient
409 availability will almost certainly interact with grazing to influence AM fungi. For example, significant
410 negative correlations between topographic-induced changes in soil nutrient availability and mycorrhizal
411 root colonization (Faghihinia *et al.*, 2020), extra-radical hyphal production (Ren *et al.*, 2018) and AMF
412 spore community composition (Murray *et al.*, 2010) under grazing have been reported suggesting

413 topography moderates the impacts of grazing on AMF. AMF may enhance plant grazing tolerance through
414 acquisition of more nutrients in more upland areas, where low- to moderate-grazing occurs and nutrients
415 are often limited. On the other hand, AMF can be less beneficial in lower lying areas where grazing is more
416 intense and competition between plant and AMF is probable due to limited photosynthetic carbon
417 (Johnson *et al.*, 2016). Consistent with this hypothesis, Faghihinia *et al.* (2020) found a greater intensity
418 and frequency of mycorrhizal root colonization under grazing in sloped sites with limited nutrient
419 availability compared to flat sites with greater nutrient availability in a temperate grassland experimental
420 system. Similarly, Bueno de Mesquita *et al.* (2018) found greater AMF root colonization at lower
421 elevations with lower availability of soil N and P in a mountain grassland.

422

423 **2.4.3. Grazing intensity**

424 Given that the number of livestock per unit area strongly impacts above- and below-ground productivity
425 and diversity (Yan *et al.*, 2013), it would be reasonable to assume that the extent of the grazing impact on
426 AMF function and community structure would largely depend on grazing intensity. Whilst overgrazing has
427 destructive and irreversible negative impacts on plant community and soil properties, undergrazing can
428 be just as harmful to grassland biodiversity and functioning through less stimulation of plant growth and
429 loss of grazing-dependent legumes and grasses (Metera *et al.*, 2010). Having said that, undergrazing is not
430 a common practice worldwide. On the other hand, moderate grazing has been identified as a benefit to
431 grassland plant and soil conditions through natural fertilization, seed dispersal, making space for annual
432 and bi-annual plant species growth and expansion, and periodic above-ground defoliation which helps to
433 regulate succession in plant communities (Metera *et al.*, 2010). However, the effects of different grazing
434 intensities on AMF is still largely unknown.
435 Most studies compared the effects of grazing on AMF abundance and diversity in grazed and ungrazed

436 plots (Murray *et al.*, 2010; Guo *et al.*, 2016; van der Heyde *et al.*, 2017), with very few assessing impacts
437 along a gradient of grazing intensity such as that ranging from light-grazing to overgrazing (Mendoza *et*
438 *al.*, 2011b; Ba *et al.*, 2012; Ren *et al.*, 2018). There is some evidence of unimodality in which the response
439 of AMF abundance to herbivory peaks at an intermediate level of grazing intensity. For examples, AMF
440 spore density increased from light to moderate grazing and decreased from moderate to extremely heavy
441 grazing in a natural meadow steppe in China (Ba *et al.*, 2012). Furthermore, an unimodal relationship
442 between the ratio of external (hyphal length density in soil) to internal AM fungal structures (mycorrhizal
443 root intensity) and grazing intensity was detected in a typical steppe grassland (Faghihinia *et al.*, 2020).
444 Given that moderate grazing can enhance plant community biomass and biodiversity (Komac *et al.*, 2015;
445 Pulungan *et al.*, 2019), positive feedbacks on below-ground root associated AMF would be expected (Ba
446 *et al.*, 2012). The impact of different grazing intensities on AMF requires further testing in different
447 ecosystems and with different AMF variables in order to determine the threshold of grazing intensity that
448 significantly alters below-ground soil microbial community composition and function in grasslands
449 ecosystems.
450

451 ## 3. Variations among empirical studies

452 Results of studies investigating the response of AMF to long-term herbivory are contradictory. For
453 example, the response of AMF root colonization to grazing is variable with either positive (Eom *et al.*,
454 2001; Hokka *et al.*, 2004; Techau *et al.*, 2004; Wearn and Gange, 2007; Nishida *et al.*, 2009), negative
455 (Wardle *et al.*, 2002; Ba *et al.*, 2012; Barber *et al.*, 2012; Birhane *et al.*, 2017; Cavagnaro *et al.*, 2018; Soka
456 and Ritchie, 2018) or neutral effects (Yang *et al.*, 2013; van der Heyde *et al.*, 2017). Grazing has been
457 shown to stimulate AMF sporulation (van der Heyde *et al.*, 2017), however, negative (Mendoza *et al.*,
458 2011a; Birhane *et al.*, 2017) and null grazing effects (Burke *et al.*, 2019) on AM fungal spore density have

459 also been documented. We suggest that in addition to the potential environmental drivers of grazing
460 impacts on AMF communities, which were discussed in this paper, the inconsistent results among studies
461 may also be due to differences in AMF variable measures.

462 AMF variables appear to respond to herbivory in different ways; while soil hyphal length density is
463 generally negatively impacted by grazing (Wang *et al.*, 2014; van der Heyde *et al.*, 2017; Ren *et al.*, 2018;
464 Vowles *et al.*, 2018), but the response of mycorrhizal root colonization and spore density to grazing is very
465 variable (Barto and Rillig, 2010; Mendoza *et al.*, 2011a; Ba *et al.*, 2012; Wang *et al.*, 2014; van der Heyde
466 *et al.*, 2017).
467 Hence, each AMF measure has its own limitation in terms of measurement and potential for yielding
468 useful insights. For example, intensity of plant root colonized by AMF is usually quantified as percent root
469 colonization. This is a relative measure, and may remain unchanged while total length of root colonized
470 may decrease following herbivory (van der Heyde *et al.*, 2017). Although classical approaches for
471 estimating percent root length colonization provide greater resolution of AMF structures, they fail to
472 describe the amount of AMF in a whole root system as they do not account for the total root length (Hart
473 and Reader, 2002), particularly in studies on individual plant species. In addition, percent root length
474 colonization does not account for how many structures were observed at each intersection meaning AMF
475 biomass deductions cannot be made. Likewise, given that spores and sporocarps are long-term survival
476 structures with the capacity of dispersal by wind and water, or locally through animal activities, with wide
477 range of age and dormancy or quiescence status, and in addition some AMF species have never been
478 detected as spores (Merryweather and Fitter, 1998a). The density of the spores in soil is unlikely to give a
479 meaningful picture of mycorrhizal community and activity.
480 Overall, differences in AMF measures in response to herbivory suggests that measuring a single variable
481 is not sufficient to elucidate this relationship and underlying mechanisms (van der Heyde *et al.*, 2019).
482 Research in this field may be advanced by including various mycorrhizal variables, especially a

483 combination of internal colonization and extra-radical hyphae, for measurement when assessing impacts
484 of environmental variables or perturbation, including grazing, on mycorrhizal communities.
485 There are also other factors which have been suggested to be influential on the magnitude and direction
486 of grazing effects on AMF. However, these factors were not sufficiently addressed in previous studies and
487 require further investigation in order to improve the management of grassland ecosystems and maintain
488 the above- and below-ground biodiversity. These include the type of herbivores due to their differences
489 in terms of browsing strategies, diet and preferential grazing of different plant species (Metera *et al.*,
490 2010). The age and body size of the grazers are also important in determining grazing preferences. Young
491 grazers and pregnant or lactating females prefer higher nutritive forages and so browse more selectively
492 when grazing (Rook *et al.*, 2004). Smaller herbivores have been shown to graze more selectively than
493 larger ones due to their small gut capacity and higher energy demands (Rook *et al.*, 2004).
494
495 Furthermore, the wide range of grassland management practices (e.g. mono- or mixed grazing, seasonal
496 grazing, selective grazing, application of fertilizers and lime, etc.) applied in many places showed shifts in
497 the plant community and soil environmental conditions (Metera *et al.*, 2010). However, how these
498 management practices affects below-ground soil biota is less clear. Further research on grazing practices
499 such as mixed- and co-grazing in conservation and management of grassland biodiversity and their impact
500 on AMF community will be interesting areas for future work.
501

502 4. Conclusions and future challenges

503 Current knowledge of the impacts of livestock grazing on mycorrhizal fungal function is inconsistent and
504 inadequate, which is mainly because of the context-dependent nature of the plant-AMF symbiosis. Here
505 we discussed a suite of biotic and abiotic drivers that regulate the response of AMF abundance and

506 community structure to grazing. Developing models, based on our framework, that integrate all of these
507 variables would be helpful to understand how the abiotic and biotic environment determines the
508 response of the plant-AMF relationship to grazing. Application of our framework of potential
509 environmental drivers allows us to make sense of the complexity and generalize on the likely impacts of
510 various grazing management approaches on AMF communities. Conducting a meta-analysis to integrate
511 the available data would be helpful to account for the variation among studies, provide quantitative
512 estimates for grazing impacts on root-associated mycorrhizal fungi and additionally to check the relative
513 importance of each biotic and abiotic variables in outcomes of the mycorrhizal symbiosis across different
514 landscapes. We also suggest that developing new investigation approaches that integrate the effect of
515 climate and grazing simultaneously, particularly at larger scales, would help to better understand the
516 degree and magnitude of grazing impact on AMF communities across climatic zones.

517 We further suggest the need for more research focusing on the impacts of different grazing intensities.
518 Understanding the response of AMF to different grazing intensities and identifying the threshold of
519 grazing intensity that significantly alters below-ground AMF would contribute to improved sustainable
520 food and forage production on grasslands by improving plant health and soil fertility. In addition, we
521 suggest that measuring a single variable is not sufficient to explain the response of AMF to grazing and
522 that the research field may be advanced by including various mycorrhizal variables for measurement.
523 Finally, and crucially, seasonality needs to be included in studies if we are to understand the mechanisms
524 underlying grazing effect on AMF and their functioning over the whole growing season. The challenge for
525 the future will be to improve knowledge and gain new insights to predict the effects of grazing on plant-
526 AMF interaction in the context of global environmental changes, with the aim to restore degraded
527 grasslands, enhance food and feed production, and maintain grassland sustainability.

528

Acknowledgments

This study was financially supported by XJTLU Research Development Fund (RDF-15-02-13).

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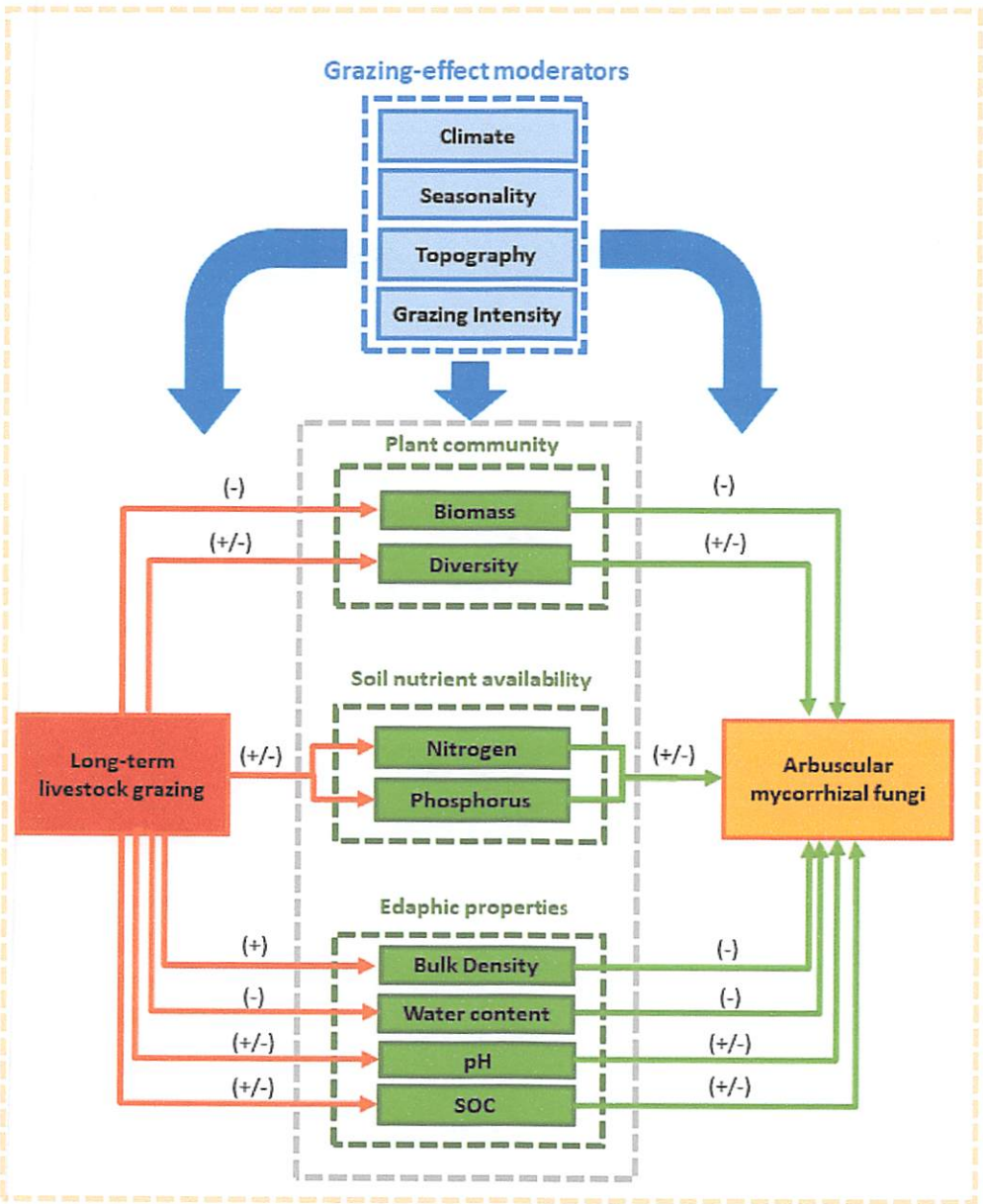

Figure 1. Conceptual framework of potential environmental drivers determining how long-term livestock grazing impacts arbuscular mycorrhizal fungi through changing the mycorrhizal environment. The possible outcome of grazing-induced change on plant community, soil nutrient availability, edaphic properties as well as AMF has been shown with + and – indicating the positive and negative effects respectively. The brown arrows indicate the direct effects of grazing on plant and soil related factors and green arrows indicate the indirect effect of grazing on AMF on each environmental variables. Blue arrows indicate the impacts of grazing-moderators (climate, seasonality, topography and grazing intensity) effects on interacting variables in the model. Dashed lines indicates that there is interaction among the variables within the boxes.

Table 1. Pathways, general outcomes and underlying mechanisms by which grazing impacts arbuscular mycorrhizal fungi (AMF) and their environment. The impacts of grazing on AMF are through the direct effects of grazing on plant community, soil nutrient availability and edaphic properties. One-way direction arrows show causal relation. + and - indicates the positive and negative effects respectively.

Pathway	Reported outcome	Potential underlying hypothesis	References
Long-term grazing effects on plant community biomass and diversity			
Grazing→ Plant biomass	-	Clipping and removing the above-ground biomass, especially apical meristems, reduces the plant photosynthetic capacity, C capture and plant productivity.	(Schönbach *et al.*, 2011;Hao and He, 2019; Piñeiro *et al.*, 2010; Yan *et al.*, 2013)
	+	Grazing-induced increase in soil nutrient availability particularly soil nitrogen can enhance plant biomass and productivity.	(Medina-Roldán *et al.*, 2012; Veen *et al.*, 2014; Chen *et al.*, 2017)
Grazing → Plant diversity	-	Loss of grazing-sensitive rare species or removal of palatable dominant plant species from species pool under grazing may decrease plant biodiversity.	(Ba *et al.*, 2012; Li *et al.*, 2015; Epelde *et al.*, 2017; Ren *et al.*, 2018)
	+	Grazing may enhance plant community biodiversity at moderate level of grazing intensity through suppressing the domination of more competitive plant species with faster growth rates and promoting the coexistence of less competitive species with lower growth rates.	(Komac et al., 2015; Pulungan et al., 2019; Wan et al., 2015)
Long-term grazing effects on soil nutrient availability			
Grazing → Soil nutrient availability	+	Adding dung and urine to the soil can enhance soil nutrient availability.	(Medina-Roldán *et al.*, 2012; Egan *et al.*, 2018; Vertès *et al.*, 2019)
	-	Grazing-caused defoliation, reduction in forage production, and litter accumulation is followed by further decreases in SOC and nutrient availability.	(Steffens *et al.*, 2008; Guo *et al.*, 2016; Hao and He, 2019)
Long-term grazing effects on edaphic variables			
Grazing → Bulk density	+	Livestock trampling and treading causes soil compaction and increases soil bulk density especially in the heavily grazed areas.	(Li *et al.*, 2012; Epelde *et al.*, 2017; Hao and He, 2019; Piñeiro *et al.*, 2010)
Grazing → Soil moisture	-	Soil compaction, diminishing plant shading effect, exposing soil to the air and increasing soil temperature reduces soil moisture and infiltration particularly in dry grasslands under grazing.	(Zhao *et al.*, 2007; Wiesmeier *et al.*, 2009; Ren *et al.*, 2018; Hao and He, 2019)
Grazing → pH	-	Grazing decreases pH via reducing soil nutrient availability particularly nitrogen.	(Ren *et al.*, 2018; Faghihinia *et al.*, 2020; Guo *et al.*, 2016)
	+	Livestock urine and faeces may increase soil available nitrogen and pH	(Hao and He, 2019)
Grazing → Soil organic carbon	-	Grazing may decrease SOC through reducing above-ground plant biomass, infiltration rates, enhancing soil compaction and SOC decomposition particularly in C3-dominated temperate grasslands.	(McSherry and Ritchie, 2013; Hao and He, 2019)
	+	Grazing may increase SOC via enhancing soil nitrogen stocks (SON), plant tissue deposition, and below-ground root litter deposition particularly in C4-dominated subtropical grasslands.	(McSherry and Ritchie, 2013; Wilson et al., 2018)

Table continued

Long-term grazing effects on AMF through impact on plant community			
Grazing → Plant biomass → AMF	-	Grazing-caused reduction in standing vegetation cover and plant photosynthetic capability reduces below-ground C allocation to root associate AMF.	(Ren *et al.*, 2018; van der Heyde *et al.*,2017)
Grazing → Plant diversity→ AMF	+	Grazing-caused promotion in plant biodiversity, particularly at intermediate intensity, may provide AMF with wide range of below-ground root exudates for AMF spores germination, hyphal extension. Further, greater diversity of host plants for AMF may facilitate the establishment of some host-specific AMF species and enhance AMF diversity.	(Ba *et al.*, 2012; Stover et al, 2018)
	-	Grazing-caused reduction in plant diversity decreases the range of below-ground plant root types and root exudates and consequently decreases the abundance and variability of the soil/root resources for AMF.	(Ba et al., 2012; Epelde et al., 2017)
Long-term grazing effects on AMF through impact on soil nutrient availability			
Grazing →Soil nutrient availability→ AMF	+	As a general pattern, grazing-induced negative impact on relative availability of soil nutrients may cause higher plant nutrient demands for regrowth and to compensate for tissue loss, leading to more investment in AMF mutualisms to obtain additional nutrients.	(Hoeksema et al., 2010; Yang et al., 2016; Guo et al., 2016)
	-	As a general pattern, grazing-induced positive impact on relative availability of soil nutrients may limit the plant investment in symbionts and result in less below-ground C allocation to AMF.	(Hoeksema et al., 2010; Yang et al., 2016)
Long-term grazing effects on AMF through impact on edaphic properties			
Grazing → pH → AMF	+	Grazing-caused positive impact on soil pH may provide a niche space for external hyphal extension.	(Mendoza *et al.*, 2011a; Hu *et al.*, 2013; Soudzilovskaia *et al.*, 2015; Guo *et al.*, 2016)
Grazing → pH → AMF	-	Soil acidification and decreasing pH under grazing condition can suppress microbial activities and growth including AMF. Further, AMF species with greater optimal pH range could disappear under soil acidic condition leading to decrease in AMF diversity	(Xu *et al.*, 2016)
Grazing → Bulk density → AMF	-	Grazing-induced soil compaction disrupts AMF hyphal networks.	(van der Heyde *et al.*,2017; Faghihinia *et al.*, 2020)
Grazing → Soil moisture→ AMF	+	Grazing-caused water limitation may constrain plant photosynthesis and primary production resulting in lower N and P demand from the plant and lower availability of photosynthate C for allocation to fungal partners	(Murray et al., 2010; Mendoza et al., 2011)
Grazing → Soil organic carbon→ AMF	+	Grazing-induced increase in root carbon allocation, fine root exudation and SOC/SON stocks could reduce the AMF functioning.	(McSherry and Ritchie, 2013; Wilson et al., 2018)

	-	Grazing-caused decrease in lower carbon allocation to roots, root and microbial biomass, and lower SOC/SON stocks could negatively impact the AMF abundance and activity.	(McSherry and Ritchie, 2013; Hao and He, 2019)